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Optimizing Photodynamic Therapy: *In vivo* pharmacokinetics of liposomal *m*-THPC in feline squamous cell carcinoma

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Optimizing Photodynamic Therapy: *In vivo* pharmacokinetics of liposomal *m*-THPC in feline squamous cell carcinoma

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ABSTRACT

Purpose: The aim of the present study was to optimize and simplify photodynamic therapy (PDT) using a new liposomal formulation (Fospeg) of the photosensitizer *m*-THPC (*meta*(tetrahydroxyphenyl)chlorin), and to reduce systemic reactions to the photosensitizer.

Experimental Design: To examine the pharmacokinetics of Fospeg, we determined tissue and plasma parameters in feline patients with spontaneous squamous cell carcinoma. *In vivo* fluorescence intensity measurements of tumor and skin were performed with a fiber spectrophotometer after intravenous injection of *m*-THPC (either Foscan® or Fospeg) in 10 cats. Blood samples, drawn at several time points after photosensitizer administration, were analyzed by HPLC (High performance liquid chromatography).

Results: None of the Fospeg treated cats showed side effects during or after drug injection. Fluorescence intensities, fluorescence ratios (tumor fluorescence divided by skin fluorescence) and bioavailability in the tumor were 2 to 4 times higher with Fospeg compared to Foscan®. Fospeg concentration in the tumor increased constantly to reach a maximum at 4 hours after injection. Plasma concentration and plasma bioavailability were about 3 times higher with Fospeg compared to Foscan® measured at the time points of highest plasma concentration. The distribution half-life was shorter with Fospeg, resulting in maximal tumor accumulation up to 5.5 times earlier. Maximal tumor accumulation and maximal fluorescence ratio with Fospeg occurred at the same time point, indicating maximal selectivity. In both groups all cats responded to therapy.

Conclusions: Fospeg was well tolerated by all cats and appears to have superior pharmacokinetic properties compared to Foscan®. The efficacy of the drug warrants further study.

INTRODUCTION

The first reports on photodynamic therapy (PDT) date back to the beginning of the last century, when researchers observed that a combination of light with hematoporphyrin induces cell death (1). In 1995 the U.S. Federal Food and Drug Administration (FDA) approved PDT as a novel form of therapy against cancer and since then, PDT has been used more frequently.

PDT includes two components combined to induce cellular and tissue effects in an oxygen dependent manner. The first is a “light-sensitive” substance called the photosensitizer. The second is light of a specific wavelength (laser light) to maximally activate the tumor-localized photosensitizer. Upon activation, a photosensitizer undergoes type I (electron or hydrogen transfer) or type II (local generation of cytotoxic singlet oxygen) photochemical reactions.

Tumor destruction associated with PDT involves three principal mechanisms (2): 1) Direct tumor cell kill (3), 2) destruction of tumor-associated vasculature (4-6) and 3) activation of an immune response against tumor cells (7, 8). A short drug-light interval allows the photosensitizer to accumulate predominantly in the vascular compartment. PDT-mediated vascular effects range from transient vascular spasm, vascular stasis and thrombus formation up to total permanent vessel occlusion and can include enhanced vascular leakiness (5). A longer drug-light interval results in maximal concentration of the photosensitizer in the tumor causing direct tumor cell destruction. This was shown recently for the second generation photosensitizer Foscan® (*meta*(tetrahydroxyphenyl)chlorin; *m*-THPC) and indicates that the *in vivo* effects occur via an indirect vascular as well as a more direct effect at different drug-light intervals (9, 10).

In order to optimize PDT, liposomes are presently being tested as carrier and delivery systems with the aim of improving the tumoritropic behavior of photosensitizers.

The present study was thus designed to optimize PDT in cat patients with spontaneous cutaneous squamous cell carcinomas using a new, liposomal formulation of *m*-THPC, called Fospeg. Pet animals with spontaneously developing cancer provide an excellent opportunity to study many aspects of cancer from etiology to treatment. Squamous cell carcinomas are common neoplasms in cats. Similar to human cutaneous squamous cell carcinoma, there is an etiologic correlation between development of the neoplasm and exposure to ultraviolet light

(12). Several studies have shown the efficacy of PDT in the treatment of feline squamous cell carcinoma (13-16).

The aim of the present study was to investigate the accumulation of Fospeg in tumor and skin *in situ* with a spectrophotometer and to define the plasma pharmacokinetics in cats. We hypothesized, that with Fospeg, a higher tumor to skin ratio and an earlier *m*-THPC concentration peak in plasma and neoplastic tissue could be achieved.

PATIENTS, MATERIALS AND METHODS

Animal Patients

Ten pet cats with histologically confirmed cutaneous squamous cell carcinoma were included in the study. Tumors were clinically staged using a modification of the World Health Organization (WHO) system (15) and included thoracic radiographs, blood analysis (hematology and organ parameters), regional lymph node aspiration and urinalysis.

All animal treatments were conducted according to the approval issued by the official veterinary authorities of the Canton of Zurich. All animal owners signed a written informed consent.

Photosensitizers

The original lipophilic formulation of *m*-THPC (Foscan®, 1.5 mg/ml, 3 ml vials) and the new liposomal formulation of *m*-THPC (Fospeg, 1.5 mg/ml, 3 ml vials) were used, kindly provided by Biolitec AG, Jena, Germany. *m*-THPC is practically insoluble in all aqueous media. The single component is of > 99% purity with its fluorescence emission peak in the red at 652 nm. The molecular weight of *m*-THPC is 680.24 Daltons.

Fospeg basically comprises DPPC (dipalmitoylphosphatidyl choline), DPPG (dipalmitoylphosphatidyl glycerol) and pegylated DSPE (pegylated distearoylphosphatidyl ethanolamine) as liposome forming compounds. The physical stability of the liposomal formulation was assessed by monitoring the particle size distribution using photon correlation spectroscopy. The mean particle size was approximately 140 nm. The degree of pegylation was 2.5-5%, and the degree of *m*-THPC was 10%. The drug is associated with the lipid membrane of

the unilamellar liposomes. The liposomal *m*-THPC formulation can be diluted with aqueous media or biological fluids without precipitation.

Drug application

All animals received 0.15 mg *m*-THPC/kg body weight out of the same production lot of either Foscan® or Fospeg. The drug was continuously injected into a cephalic or femoral vein over 10 minutes. Cats that were given Foscan® were premedicated with Clemastin (Tavegyl®, Novartis Consumer Health Schweiz AG Bern; 0.05 mg/kg), a H1-receptor antihistamine. Cats receiving Fospeg were not premedicated, in order to observe systemic reactions if any.
For all measurements, the time t = 0 corresponded to the end of the injection.

Spectrophotometric fluorescence measurements

In vivo fluorescence measurements were performed non-invasively a) in normal skin (unpigmented) and b) in the tumor every hour for the first 10 h and then 16, 24, 36 (Foscan® group only), 48 and 72 h after drug injection. A fiber spectrometer (optical biopsy system, OBS, kindly provided by Dr. Martin O'Dwyer, University of Glasgow, UK and Biolitec, Jena, Germany) was used. This instrument is composed of a 405 nm laser diode excitation source, an optical fiber, and a spectrometer. Emission from the tissue was collected and after suitable filtering, the spectra from 450 to 700 nm were displayed (integration time 500 ms). Intensity values at 652 nm were recorded. Photobleaching at the surface was not relevant due to short acquisition times and a low power at the end of the fiber (30 μW). The tip of the fiber was held perpendicular to the surface of the tissue exerting constant pressure to assure contact. At each location 3 individual measurements were obtained and the mean value was determined. The spectrophotometer was calibrated at zero (dark current) prior to each measurement.

Photosensitizer concentration in plasma/urine

To determine the plasma concentration of *m*-THPC, blood samples were obtained 1, 3, 6, 8, 16, 24, 48 and 72 hours after application of the photosensitizer.

The 1 - 1.5 ml blood samples were collected in sterile CTAD tubes (Becton Dickinson AG, CH-4002 Basel, Switzerland). The tubes were immediately centrifuged for 30 min at 2'500 g and 4°C. The plasma supernatant was stored at –80°C. The plasma *m*-THPC concentration was measured by HPLC (Biolitec AG, Jena, Germany). A weighted sample of 20 mg was blended with 1.5 ml of DMSO/methanol (5:3, $^{v}/_{v}$) and agitated for 12 hours at 60°C. Afterwards, the samples were centrifuged for 5 min at 13'000 g and 1 ml of the clear supernatant was removed for HPLC analysis (Gold System Module 168, Beckman + Fluorescence detector RF-10A XL, Shimadzu, Column: "LiChroCART 250-4" with Purospher STAR RP-18 end capped; 5 µm; (Merck), Guard column: "LiChroCART 4-4" with Purospher STAR RP-18e; 5 µm (Merck), Temperature: 30 °C, Mobile phase: acetonitrile: H_2O + 0.1 % trifluoroacetic acid = 57.5% (v/v) : 42.5% (v/v), Flow rate: 1 ml/min.). The fluorescence wavelength was set at 410 nm for excitation and 653 nm for emission. The plasma *m*-THPC concentration was calculated from a calibration curve constructed by plotting the peak height values of *m*-THPC standard solutions versus their concentrations.

Urine samples were taken at different time points after injection and analyzed by HPLC for potential *m*-THPC content.

Treatment

PDT of the spontaneous cutaneous carcinomas in client owned cats was performed under anesthesia 48 h after Foscan® injection and 16 h after Fospeg injection. The interval of 48 h for Foscan® was based on previous experience in cats (13). For Fospeg, the shorter interval of 16 h was based on results from laboratory mice, that indicated a more rapid metabolism for the liposomal formulation (Dr. Susanna Gräfe, Biolitec, unpublished data).

A 652 nm diode laser (Applied Optronics Corp., South Plainfield, NJ) was used as light source. The light was delivered using a quartz optical fiber with a micro lens at the tip (Medlight SA, Ecublens, Switzerland). Non-contact surface illumination of the entire tumor area plus a security margin of 5 mm was implemented. The power at the end of the fiber was measured by a calibrated power meter at 652 nm and the laser was adjusted to obtain a 0.05 W/cm^2 non-

thermal power density on the irradiated surfaces. A dose of 10 J/cm^2 was delivered and the treatment time (200 sec) was controlled with the laser`s built in countdown timer.

Anesthesia was induced by intravenous application of Buprenorphin (Temgesic®, ESSEX Chemie AG, 6005 Luzern, Switzerland; 10µg/kg), Midazolam (Dormicum®, Roche Pharma AG, Reinach, Switzerland; 0.2 mg/kg iv) and Propofol (Propofol®, Fresenius Kabi AG, Stans, Switzerland). Propofol was given until tracheal intubation was possible. Anesthesia was maintained with Isofluran (Forene®, Abbott AG, Baar, Switzerland; 1-3% in oxygen). Hemoglobin oxygen saturation and heart rate were monitored continuously throughout anesthesia with a pulse oxymeter, and values were recorded before and during illumination. Ringer's lactate solution was administered intravenously at 4 ml kg^{-1} h^{-1}.

Post-treatment the cats received Buprenorphin (10 µg/kg) every 8 h for 48 hours. The following 3 weeks they received Piroxicam (Pirocam®, Spirig Pharma AG, 4622 Egerkingen, Switzerland; 0.3 mg/cat) once a day as pain medication. If necessary, amoxicillin and clavulanic acid (Synulox®, Pfitzer AG, 8048 Zürich, Switzerland; 12.5 mg/kg twice a day) was given.

Toxicity

Acute toxicity was assessed during and after photosensitizer administration. Blood and urine samples were taken 72 h after injection of the photosensitizer to rule out an effect of the drug on organ parameters. Tissue reactions were assessed during, 1 hour and 4 days after light treatment. Toxicity was scored according to CTCAE v3.0 (Common Terminology Criteria for Adverse Events).

Statistical analysis

Pharmacokinetic values were obtained by using Pharmacokinetic (PK) Functions for Microsoft Excel. Description of data is given by mean and median (± standard deviation; SD). Stat View 5.0.1 software was used for statistical analysis. Data were investigated graphically by box plots. In order to investigate differences between both drugs with respect to tissue fluorescence and

plasma parameters, the Mann-Whitney-U-test was applied. P values < 0.05 were considered significant.

RESULTS

Animal patients

All patients included in the study were European short hair cats. Four of the patients were female spayed, 6 were male neutered. There was no statistically significant difference concerning gender, age or weight between the two groups. All tumors (n=10) were classified as squamous cell carcinoma by histopathological examination. All tumors were located on the head with the majority of tumors occurring on the nasal planum (n=6). Most of the treated squamous cell carcinomas were low stage tumors (T1a-T2a; n=7). None of the cats had evidence of metastatic disease (N0M0).

Spectrophotometric fluorescence measurements

Patients receiving Foscan® showed mean and median maximal tumor fluorescence intensities of 160.88 and 154.89 (relative units), respectively. These maxima were seen between 36 and 48 h after injection (Table 1). In patients treated with Fospeg, the mean and median maximal *m*-THPC fluorescence intensities in the tumor were 275.67 and 279.00 (relative units), respectively. In this group, maxima were reached 5 to 10 h after injection (Table 1). The fluorescence intensity maxima were not significantly different (P= 0.139), even though a distinct difference is obvious (Figure 1). The intensity increased rapidly over the first 4 h to reach a maximum and a plateau phase 4 to 6 h after Fospeg injection. The time point of intensity maxima differed significantly (P= 0.015) between the two groups. The fluorescence ratio, defined as the mean fluorescence intensity of the tumor divided by the mean fluorescence intensity of normal skin at a given time point, was calculated for each individual animal. For Foscan®, these ratios ranged from 1.79 to 4.81 (mean and median values of 3.02 and 2.45, respectively). For Fospeg, the ratios ranged from 3.47 to 18.34 (mean and median value of 10.75 and 11.98, respectively). The fluorescence ratio was higher for Fospeg compared to

Foscan® (Figure 1), although statistical significance was not achieved (P=0.053). Highest ratios were reached much later after injection of Foscan® than after injection of Fospeg (Table 1); with Fospeg a rapid increase during the first 5 to 10 h could be observed. For Fospeg only, the time point of the maximal fluorescence intensities and the time point of the highest tumor to skin ratio were identical.

The mean and median bioavailability in the tumor, calculated for the first 16 h after injection (area under the curve; AUC_{16}) was significantly higher for Fospeg than for Foscan® (P=0.030; Figure 2). The bioavailability in the skin was similar for both photosensitizers (P=0.305). The AUC_{16}-ratio, calculated from the tumor values divided by the skin values, for Foscan® resulted in a mean and median of 2.00 and 1.55, whereas the AUC_{16}-ratio for Fospeg showed mean and median values of 6.94 and 6.50, respectively (P= 0.053; Table 1).

Photosensitizer concentration in plasma/urine

In the Foscan® group, mean and median maximal *m*-THPC levels of 431.38 and 458.81 ng/ml plasma were reached during the first 8 h after injection. During this time range, *m*-THPC levels remained constant or increased slightly. Afterwards, the photosensitizer concentration decreased to reach a level near baseline 48 h after injection. For Foscan®, the calculated distribution half-life (hour 0-16) resulted in mean and median values of 46.74 and 44.62 h, respectively. The calculated elimination half-life (hour 16-72) showed mean and median values of 20.64 h and 20.32 h, respectively (Table 2). In the Fospeg group, significantly higher mean and median maximal *m*-THPC levels were found (1317.08 and 1523.30 ng/ml plasma, respectively), compared to the Foscan® group (P= 0.017). The highest *m*-THPC plasma levels were seen at the first time point measured, followed by an immediate and rapid disappearance of the photosensitizer signal (Figure 3). For Fospeg, the calculated distribution half-life (hour 0-16) was significantly shorter (P=0.017), resulting in mean and median values of 9.36 and 7.95 h. The calculated elimination half-life (hour 16-72) resulted in mean and median values of 22.90 and 19.83 h, which was comparable to the values obtained with Foscan® (P= 0.732; Table 2). Plasma drug concentrations returned to near baseline 48 h after injection.

The mean and median bioavailability, calculated at 16 and at 72 h, was much lower in the Foscan® group compared to the Fospeg group (P= 0.053 and P= 0.087, respectively; Table 2). *m*-THPC could not be detected in the urine at any of the time points examined after injection.

Treatment response

All 3 cats in the Foscan® group had a complete response to therapy. No tumor recurrence could be observed so far. In the Fospeg group 4 cats had a complete, 3 a partial response. Two of these three cats had tumor recurrences and no further therapy was done, while 1 cat was retreated with PDT and then had a complete response. No tumors have recurred in the cats with a complete response. The longest follow up time in both therapy groups, however, is only 380 days.

Toxicity

During or shortly after Foscan® injection, 2 of the 3 cats showed side effects such as tachypnoe, salivation and excitation, even though they were premedicated.

The Fospeg injection was well-tolerated by all cats. None of the Fospeg treated animals showed signs of acute toxicity, such as vomiting, diarrhea, salivation, tachypnoe, excitation or death. There was no obvious difference in skin reaction between the 2 groups. Only 1 cat in the Fospeg group (patient 7), which had extremely high skin fluorescence intensities, developed sunburn-like reactions on the face about 10 days after injection which healed uneventfully.

DISCUSSION

PDT has the potential to be a powerful treatment modality for cancer, either applied solitary or in combination with chemotherapy (17), surgery (18), radiotherapy (19) or other strategies, such as hyperthermia (20). However, low selectivity, inconveniently long drug-light intervals, and prolonged generalized photosensitivity are problems encountered with this therapy modality. Modifying the photosensitizing moiety through its physicochemical properties may improve PDT.

In the present study, a new formulation of a commercially available photosensitizer has been studied to address the following hypotheses: the liposomal formulation 1) allows a shorter drug-

light interval, 2) results in a higher tumor to skin ratio and 3) shows an earlier plasma peak, compared to the conventional formulation.

Maximal tumor fluorescence intensities with Fospeg were nearly twice as high compared to Foscan®. This is important clinically, as the drug dose needed could probably be lowered. A lower drug dose would result in a shorter generalized photosensitivity of the patients. With Fospeg, the time of maximal fluorescence intensity in the tumor was shown to be significantly earlier than with Foscan® (P= 0.015). These results suggest that the drug-light interval could be reduced by a factor of 5.5, representing a second relevant advantage for clinics and fulfilling the first hypothesis. For Fospeg, the time point of maximal tumor accumulation was in accordance with the time of maximal tumor to skin ratio. For Foscan®, this was not the case in the present study. In previously published work using Foscan®, a drug-light interval of 4 to 12 h resulted in extensive skin and muscle necrosis (21, 22), indicating a low tumor to normal tissue ratio at this early time point. In contrary, no obvious changes in normal tissues were observed at any drug-light interval when pegylated *m*-THPC was used (23). Results of our study clearly show much higher tumor to skin ratios in spontaneous feline squamous cell carcinoma with the liposomal drug. In general, the uptake of pegylated photosensitizers is enhanced due to an increased vascular permeability of tumor vessels. In addition, tumor tissue lacks a functional lymphatic system, therefore, extravasated macromolecules can not return efficiently to the central circulation. By showing a 4 times higher maximal tumor to skin ratio for Fospeg compared to Foscan®, we proved our second hypothesis. A mean fluorescence ratio of about 12 indicates a distinct selectivity of the new formulation. The lack of statistical significance may be due to the fairly small sample size in each group, although a clear trend was seen (P=0.053).

The results reported here point at differences of 3 to 4 orders of magnitude for Fospeg compared to Foscan® in terms of changes in AUC, which means a significantly higher bioavailability for the new liposomal *m*-THPC formulation (P=0.030). This suggests again the possibility to decrease the typical administered dosage of *m*-THPC. Also, the AUC_{16}-ratio for Fospeg is about 4 times higher than for Foscan®, showing a much higher overall accumulation in the tumor than in the skin.

For Fospeg, the maximal plasma *m*-THPC levels were about 3.5 times higher than for Foscan® (P= 0.017). With Fospeg, the maximum plasma concentration was attained at the first time point measured, while for Foscan® maximum plasma concentrations were seen about 6 h after injection. For the lipophilic *m*-THPC a delayed concentration maximum has been shown in the plasma of humans, dogs, cats, rabbits and nude rats (24). Reasons for this phenomenon, such as aggregation of hydrophobic substances such as Foscan®, can be substantially decreased by using liposomal formulations. Therefore, our third hypothesis of an earlier plasma peak after Fospeg injection was confirmed. The mean half-lives calculated for Foscan® as well as the shape of the graphs are similar to those of a previous study in cats (25). The shorter distribution half-life of Fospeg could be the reason for the earlier maximal photosensitizer accumulation in the tumor. We did not see any complication during injection of Fospeg. During and after the injection of Foscan®, 2 of 3 cats showed side effects such as tachypnoe, salivation and excitation. Premedication was used because in previously treated cats these symptoms were seen at our institution. These side effects led to the discontinuation of Foscan® after 3 cats. Cats in the Fospeg group were not premedicated because this could have masked even weak side effects. The owners were told to keep the cats away from direct sunlight for another 10 days. The one cat that developed pronounced skin reactions was, in addition to a very low tumor to skin AUC_{16}, housed at high altitude and went outside 1 week after injection.

In this study we examined tumor, skin and plasma drug levels in order to generate an optimized and simplified PDT protocol. In a recent report (10) no significant correlation between tumor drug level and PDT response, but between plasma drug level and tumor response was found for Foscan®-PDT. This suggests that illumination in clinical PDT should be performed at highest plasma levels, targeting the vasculature more than the tumor cell directly. In another study, Foscan®-PDT had two peaks of activity: an early effect on tumor vasculature synchronous to the plasma peak level, followed by a late direct effect at maximum tumor accumulation (9). After having determined the tumor and tissue peaks in the feline species, our next step will be to

compare the PDT outcome of the cats treated to date with cats treated optimally, i.e. at the time of highest Fospeg tumor accumulation versus cats treated at the time of the plasma peak.
All patients of both groups responded to the treatment showing either a complete or a partial tumor remission.
Due to the small patient number and the fact that we just now determined the tumor and plasma peaks for Fospeg in the feline species, a true comparison between the effectiveness of the 2 formulations is not yet possible. Fospeg appears to be at least as effective as Foscan®.

In conclusion, we have shown that the new, liposomal formulation of *m*-THPC is a safe drug, causing no noticeable acute side effects in any cat in the present study. With Fospeg, important progress for PDT could be achieved. The combination of a higher selectivity and significantly earlier tumor and plasma peaks will result in a more efficient and eventually more effective PDT protocol.

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Figure 1 Tumor and skin fluorescence intensities as a function of time after injection of either Foscan® or Fospeg (median values of all patients; error bars indicate the standard deviation, SD); in the right upper corner the lower three plots are shown as a magnified view with an enlarged scale for the fluorescence intensities

Figure 2 Bioavailability of either Foscan® (=1) or Fospeg (=2) in the tumor during the first 16 h after injection (Area under the curve after 16 hours; AUC_{16}): Box plot investigation

Figure 3 Plasma *m*-THPC concentration as a function of time after injection of either Foscan® or Fospeg (median values of all patients; error bars indicate the standard deviation, SD)

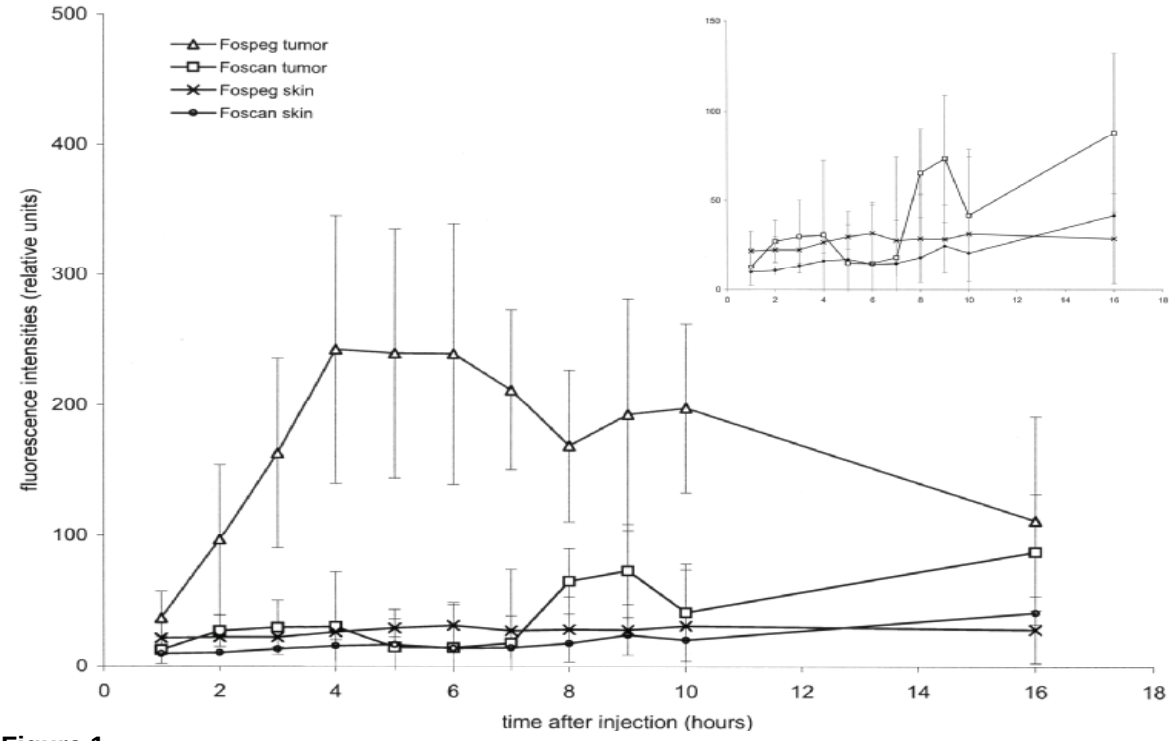

Figure 1

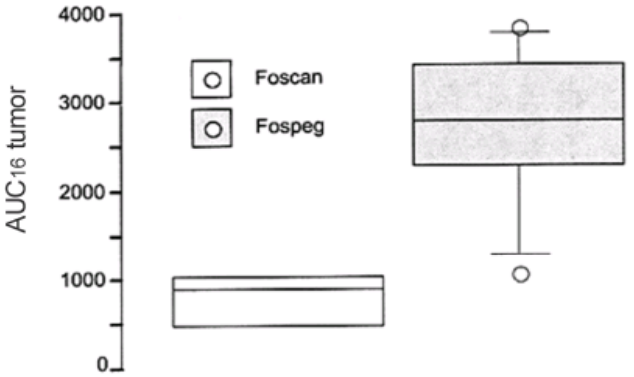

Figure 2

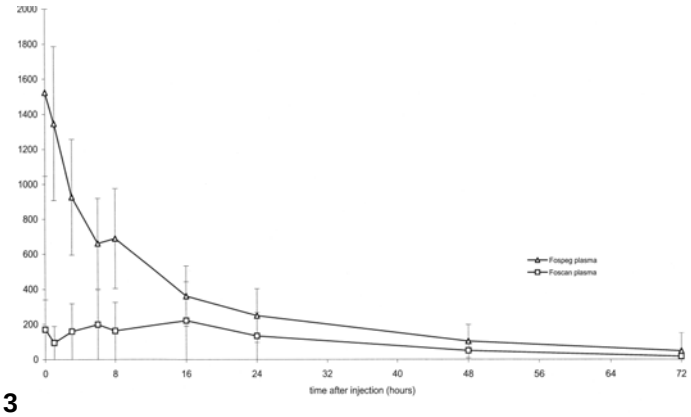

Figure 3

Table 1 Tissue fluorescence parameters for Foscan® (Patient 1-3) and Fospeg (Patient 4-10)

Patient	Maximal fluorescence intensities (tumor)	Time of maximal fluorescence intensities (tumor) (h)	Maximal tumor to tissue ratio	Time of maximal tumor to tissue ratio (h)	AUC_{16}* tumor	AUC_{16}* skin	AUC_{16}* tumor/ skin
1	78.72	36	1.79	4	334.41	232.59	1.44
2	249.03	36	4.81	6	1081.73	359.29	3.01
3	154.89	48	2.45	24	900.73	582.00	1.55
Mean	**160.88**	**40.00**	**3.02**	**11.33**	**772.29**	**391.29**	**2.00**
Median	**154.89**	**36.00**	**2.45**	**6.00**	**900.73**	**359.29**	**1.55**
SD	**85.31**	**6.93**	**1.59**	**11.02**	**389.87**	**176.89**	**0.88**
4	279.00	6	18.34	6	3559.33	391.33	9
5	407.33	10	11.98	10	4332.50	420.50	10.30
6	234.67	10	7.82	10	1989.00	478.33	4.16
7	212.00	7	3.95	7	2244.17	1188.50	1.89
8	99.33	7	3.47	7	4332.50	420.50	10.30
9	350.67	5	13.66	5	2428.50	376.50	6.45
10	346.67	6	16.00	6	2781.50	427.72	6.50
Mean	**275.67**	**7.29**	**10.75**	**7.29**	**3095.38**	**529.05**	**6.94**
Median	**279.00**	**7.00**	**11.98**	**7.00**	**2781.50**	**420.50**	**6.50**
SD	**103.96**	**1.98**	**5.82**	**1.98**	**980.48**	**292.55**	**3.18**

* Area under the curve 16 hours after injection

Table 2 Plasma parameters for Foscan® (Patient 1-3) and Fospeg (Patient 4-10)

Patient	C_{max} [§] (ng/ml plasma)	T_{max} [¶] (h)	$t_{½}$ distribution [#] (h) (hour 0-16)	$t_{½}$ elimination [##] (h) (hour 16-72)	Elimination Rate Constant (hour 0-16)	Elimination Rate Constant (hour 16-72)	AUC_{16} [*] (ng x h x ml^{-1})	AUC_{72} [**] (ng x h x ml^{-1})
1	458.81	8	56.86	20.32	0.01	0.03	4974.64	7765.53
2	238.12	6	44.62	23.25	0.02	0.03	3390.01	7039.13
3	597.22	6	**38.75**	18.35	0.02	0.04	8217.56	19193.93
Mean	**431.38**	**6.67**	**46.74**	**20.64**	**0.02**	**0.03**	**5527.40**	**11332.86**
Median	**458.81**	**6.00**	**44.62**	**20.32**	**0.02**	**0.03**	**4974.64**	**7765.53**
SD	**181.12**	**1.15**	**9.24**	**2.47**	**0.00**	**0.00**	**2460.79**	**6817.56**
4	1690.10	0	6.97	22.35	0.10	0.03	13341.84	22050.53
5	854.92	0	6.45	17.71	0.11	0.04	5975.30	9418.44
6	693.74	0	9.61	14.36	0.07	0.05	6591.53	11190.45
7	1757.00	0	6.37	19.83	0.11	0.03	11294.70	17921.64
8	1523.30	0	14.42	44.66	0.05	0.02	17593.76	39756.32
9	1789.45	0	7.95	13.55	0.09	0.05	13736.35	26490.77
10	911.04	0	13.72	27.82	0.05	0.02	10671.77	22260.78
Mean	**1317.08**	**0**	**9.36**	**22.90**	**0.08**	**0.03**	**11315.03**	**21298.42**
Median	**1523.30**	**0**	**7.95**	**19.83**	**0.09**	**0.03**	**11294.70**	**22050.53**
SD	**477.03**	**0**	**3.41**	**10.76**	**0.03**	**0.01**	**4095.35**	**10196.85**

[§] Maximal plasma concentration
[¶] Time point of maximal plasma concentration
[#] Distribution half-life
[##] Elimination half-life
[*] Area under the curve 16 hours after injection
[**] Area under the curve 72 hours after injection

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